International Standard Serial Number : 0976-1381

Deccan J. Natural Products 2(3): July-September 2011

DECCAN PHARMA JOURNAL SERIES

ARMS Online Publications

www.deccanpharmajournals.com

(Research Article)

Received; accepted

ANTI-DIABETIC ACTIVITY OF *CASSIA AURICULATA* (LINN) WALL, SEEDS ON STREPTOZOTOCIN INDUCED DIABETIC RATS

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Keywords:
Cassia auriculata, Antidiabetic, Metformin, Streptozotocin

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ABSTRACT

Objective: To evaluate an antidiabetic potential of seeds of *Cassia auriculata* (Linn).

Material and Methods: In the present study, the seeds of *Cassia auriculata* (Linn) were screened for antidiabetic activity. The seeds of *Cassia auriculata* (Linn) were subjected to hot continuous extraction (soxhlet) with ethanol as universal solvent, and fractionation was carried out to obtained pet.ether and chloroform fraction. Aqueous extract was prepared by cold maceration. After qualitative phytochemical investigations, all the extracts and fractions were subjected for antidiabetic activity in streptozotocin induced diabetes in rats. All the extracts and fractions were given orally at a dose of 300mg/kg body weight. Metformin was used as standard drug (250mg/kg body weight p.o.).

Result: The alcoholic as well as aqueous showed significant antidiabetic activity as compared to streptozotocin induced diabetes in rats.

Introduction –

The plant *Cassia auriculata* (Linn) (Cesalpiniaceae) is a fast growing branched tall, evergreen shrub with reddish brown branches. It is common plant in Asia and has been widely used in traditional medicine and has a potent adjunct in the treatment of rheumatism, conjuctivis and diabetes. The seeds are also been used in dysentery, ophthalmia, and yellow flowers are commonly used for the treatment of skin disorders and body odour. The indigenous peoples uses various parts of the plants for treatment of diabetes mellitus and it is widely used in Ayurvedic medicine as a 'Kalpa drug' consist of five parts of shrub (roots, leaves, flowers, barks and unripe fruits) which are taken in equal quantity, dried and then powdered to give 'Avarai Panchanga Choornam' for the control of sugar level and symptoms such as polyuria and thirst in diabetes. (1). The literature survey showed that decoction of leaves, flowers and seeds of *C. Auriculata* mediated the antidiabetic effect (2). The literature report shows that a very little work has been done with respect to *Cassia auriculata* (Linn) seeds other than its hypoglycemic effect. Hence the present investigation has undertaken to evaluate the effects of ethanol, aqueous and fractions of ethanolic extracts of *Cassia auriculata* (Linn) for antidiabetic activity in streptozotocin induced rats. The effects of the extract was also been compared with that of the standard drug i.e. metformin, a well known antidiabetic drug.

The various extracts of the dried seeds of *Cassia auriculata* is used traditionally for the treatment of diabetes. Aqueous and ethanolic extracts of *Cassia auriculata* Wall seeds are significantly proved to possess anti-diabetic activity.

Materials and methods –

Plant material

The seeds of *Cassia auriculata* was collected from local areas of Sahyadri Hill ranges of Pune, identified and authenticated from Botanical Survey of India, Pune. A specimen voucher (No.DGYCASA1) of the same is deposited in Dept. of Pharmacognosy, Sharadchandra Pawar College of Pharmacy, Otur (Dumberwadi).

Extraction of plant material

The collected seeds of *Cassia auriculata* was shade dried, powdered and extracted with ethanol as a solvent using a Soxhlet extractor (25 cycles) at 5 batches. After exhaustive extraction, the collected extract was dried under reduced pressure using rotary flash evaporator and dried at room temperature to obtain the residue. The part of extract was used for fractionation with pet ether and chloroform.

The aqueous extract was being obtained by subjecting the drug to the maceration process carried out for 7 days using the distilled water with occasional stirring and concentrating the supernant liquid to the small volume under the reduced pressure and then evaporated to the dryness.

The aqueous, alcoholic, pet ether and chloroform extracts of *Cassia auriculata* were subjected for further anti-diabetic studies.

Animals

Male Swiss albino mice weighing between 20-25gm and male albino wistar rats weighing 150-200 gm were used for this study. The animals were obtained from animal house, Raj Biotech, Pune. On arrival, the animals were placed randomly and allocated to treatment groups in polypropelene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{0}C and relative humidity of 30-70%. A 12:12 light: day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pelleted rat chaw diet. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (1197/08c/CPCSEA) and were in accordance with the guidelines of the CPCSEA.

Antidiabetics screening

Acute toxicity study

The oral acute toxicity was carried out as per OECD guidelines. 300mg/kg b.w of each extract was taken as effective dose for evaluation of antidiabetic activity.[3]

Preparation of dose

The alcoholic, aqueous extract and fractions of pet. ether and chloroform of *Cassia auriculata* 300mg/kg b.w. were

formulated as suspension in distilled water using 1% tween 80 as suspending agent. Since 1% tween 80 has negligible effect on normal blood glucose level (BGL). The strength of the suspension was according to the dose administered and was expressed as weight of dried extract.

Preparation of standard drug

Metformin (250mg/kg b.w.) was used as the standard drug for evaluating the antidiabetic activity. The metformin was prepared by dissolving it in 0.9% normal saline solution. The metformin was taken from Micro Lab, Bangalore. Strength of the suspension was adjusted to 20mg/0.1ml b.w.

Induction of Diabetes:

Steptozotocin (50mg/kg) b.w. in 0.1M citrate buffer, pH 4.5 was injected intarperitonially to induce hyperglycemias.[4] The animals were considered diabetic when the blood glucose level (BGL) raised beyond 150mg/dl of blood and this condition was observed at the end of 72hr. after inducing streptozotocin. The animals were segregated into six groups of six rats each taking into consideration the diabetic BGL.

Methodology:

Before starting of the experiment, animals were separated according to their body weights. The animals were segregated into seven groups of six rats each, taking into consideration of diabetic blood sugar level. The alcoholic, aqueous and the fraction of pet. ether and chloroform of *Cassia auriculata* (300mg/kg b.w./oral) and the metformin (250mg/kg b.w./oral) and saline treated were orally administered, for every 24hrs. for a period of 7 days to rats using oral gauze.

Group I - control.

Group II - received STZ 50mg/kg. b.w. by i.p

Group III- received STZ 50mg/kg. b.w. by i.p + metformin (250 mg/kg), p.o.

Group IV- received STZ 50mg/kg. b.w. by i.p + acoholic extract of

Cassia auriculata (300 mg/kg), p.o

Group V - received STZ 50mg/kg. b.w. by i.p + aqueous extract of

Cassia auriculata (300 mg/kg), p.o

Group VI - received STZ 50mg/kg. b.w. by i.p + Chloroform fraction of

Cassia auriculata (300 mg/kg), p.o

Group VII - received STZ 50mg/kg. b.w. by i.p + pet ether fraction of

Cassia auriculata (300 mg/kg), p.o

The blood was collected in 1.5ml fluoride vial bottles. The blood glucose level was estimated in plasma on 1st, 3rd, 5th and 7th day of treatment. The OGTT was performed on the 7th day of the treatment. The blood samples were collected through retro-orbital puncture by micro hematocrit capillaries from fasted animals (12hrs.) under light ether anaesthesia.

Estimation of OGTT:

Estimation of the glucose for OGTT was carried out on the 7th day after treatment. The animals were fasted for 16hrs. After end of treatment, the dose of glucose (2gm/kg b.w.) was administered and the blood samples were collected at an interval of 0 hr., 1st hr., 3rd hr., 5th hr., and 7th hr. The blood sample was collected in fluorides tube and the plasma was separated and analyzed for the glucose (RFCL, Ltd.), cholesterol (RFCL, Ltd.), triglyceride (Span Diagnostic Ltd.) and HDL-cholesterol (RFCL, Ltd.) using diagnostic reagent kit.

Histopathological Evaluation:

Tissue samples from the pancreas were fixed in 10% buffered neutral formalin, embedded in paraffin screened at 5µm and stained with hematoxylin-eosin and periodic acid-schiff.

Statistical analysis-

The data's were expressed as mean ± SEM. Obtained from the number of experiment (n). One-Way ANOVA with Dunnet' post test was performed using GraphPad Prism version 5.00 for windows.

Results-

Blood glucose level (BGL) in different groups

In the normal control group the BGL was about 80mg/dl throughout the study.

The STZ- induced group has sustained BGL from 204.2 to 248 mg/dl during the same period. In other groups in which extracts were given, ranges of the BGL are given for the blood collected on 1st to 7th day. Details for 0,1st, 3rd 5th and 7th day are in table no.1

1. Std.drug- metformin - 247 to 86.01
2. Alcoholic extract - 228 to 93.19
3. Aqueous extract - 225 to 99.54
4. Pet.ether fraction - 195.0 to 89.57
5. chloroform fraction – 217.8 to 199.1

The STZ induced group was compared with groups treated with the extracts. The fall in blood glucose level significantly ($p<0.0001$) in alcoholic extract, aqueous extract and pet.ether fraction treated groups. However the extract and fraction showed significant fall of BGL. Overall the test drug is effective in reducing BGL in STZ induced rats.

Oral Glucose Tolerance Test (OGTT)

STZ induced group of diabetic rats are treated with, aqueous extract, alcoholic extract and pet.ether fraction have shown the considerable reduction in BGL. The results are shown in table no.2. The reduction in glucose level is significant ($p<0.0001$) in the treated animals from its 1hr, 3hr, 5hr and 7 hr of drug administration. The treatment of std.drug metformin (250 mg/kg. b.w) produced at 7 hr.

However pet.ether fraction has shown maximum reduction in BGL at 3rd hour and its faster when compared with alcoholic and aqueous extract. So the pet.ether fraction alcoholic extract and aqueous extract showed reduction in BGL which is near to std.drug metformin.

Plasma lipid profile:-

The lipid profiles in normal and experimental rats are depicted in table. 3. In STZ induced diabetic rats there was significant increase ($p<0.001$) in total cholesterol and triglycerides and decrease in high density lipo protein (HDL) cholesterol in plasma with compared to normal control. The plant extracts used (aqueous, alcoholic and two fractions pet ether and chloroform) in the study significantly ($p<0.001$) decrease the cholesterol and triglyceride and significantly ($p<0.001$) increase

HDL cholesterol. This indicates that plant extracts had favorable effects on lipid metabolism of diabetic rats. Derangement of glucose, fat and protein metabolism in diabetes result in the development of hyperlipidemia (5) significant lowering of total cholesterol in alcoholic extract (33.29± 5.075), aqueous extract (38.97± 1.942) rise in HDL cholesterol. is very desirable biochemical state for the prevention of atherosclerosis and chemical condition.

Figure A and figure B represent the islet of langerhans for normal and streptozotocin-induced diabetic rats respectively. Comparison of A and B clearly indicates the increase in the numbers of β-cells in diabetic rats. As it is evident that in figure B the islets are large are irregular shaped, most of cells of islets are small, degenerated and dark with scanty cytoplasm in all treated group of animals, severe vaculation and degeneration are present in the β-cells of numbers of islets.

However compared to streptozotocin induced diabetic rats histopathological examination of aqueous, alcoholic and chloroform fraction treated diabetic rats revealed bring to normal position. The presence of β-cells (figure C, D and E) and thus restoration of normal cellular population size of islets with hyperplasia by metformin (figure. F)

Discussion –

Traditionally plant remedies have been used for centuries in the treatment of diabetes but only few are scientifically evaluated. STZ is well known for its selective pancreatic islets cell toxicity and has been extensively used to induce diabetes mellitus in animals. (6)

The present study was undertaken to assess the anti-hyper glycemic activity of alcoholic, aqueous extract, pet.ether and chloroform fraction of *Cassia auriculata* seeds. Previously it was reported that different parts of *Cassia auriculata* plant possess anti-diabetic efficacy. (7)

Several authors reported that flavonoids, sterols/triterpenoids, phenolic acids are known to be bio-active anti-diabetic principles. (8, 9).

In the present study it has been observed that hypo-glycemic effect

extended prominently by alcoholic, aqueous extract and pet.ether fraction are quite effective which is almost corresponds action of std.drug metformin.

In the present acute study as well as OGTT, it has been observed that aqueous, alcoholic extract and pet.ether fraction were highly significant ($p<0.0001$)

It is concluded from the data that aqueous extract, alcoholic extract and pet.ether fraction reduce the BGL, cholesterol and triglycerides and showed increase in HDL cholesterol. Significant lowering of total cholesterol and triglyceride is very desirable bio-chemical state for prevention of diabetes and its complication (10)

Our histopathological investigation along with the biochemical evaluation suggests the possibility of the islets regeneration upon plant extract treatment. Further research is required to explore exactly the mechanism of islet regeneration by the plant extract.

References –

1. Brahmachari HB, Augusti KT, Hypoglycaemic agents from Indigenous plants. J. Pharm. Pharmacol. 196113: 381-85.
2. Shrotri DS, Aiman R, Indian Journal of Physiology and Pharmacology, 1960;4:1-16
3. OECD/OCDE, Guidelines for the testing of chemicals. Revised draft guidelines 423: Acute oral toxicity-Acute toxic class method, revised document. 2000 Oct.
4. Pari L, Latha M. Singapore Med J, 2002; 43 (12): 617.
5. Nandhakumar J, Malini A. Anti-diabetic activity of methanol leaf extract of *Costus pictus* D.Don in Alloxan-induced Diabetic rats. J of Health Sci, , 2207;53(6): 655-63
6. Prasad SK, Kulshreshtha A, Taj N. Qureshi, Antidiabetic Activity of Some Herbal Plants in Streptozotocin Induced Diabetic Albino Rats. Pakistan J of Nutri, 2009;8 (5): 551-57.
7. Shrotri DS, Kelkar M, Deshmukeh VK, Aiman R. Investigations of the hypoglycemic properties of *Vinca rosea, Cassia auriculata* and *Eugenia jambolana*. Ind JMed Res 1963; 51: 464- 67.
8. Oliver – Bever B. Medicinal plants in tropical West Africa, Cambridge University press, London; 1986:245 – 67
9. Atta-Ur-Rhemann, Khurshid Zaman. Medicinal plants with hypoglycemic activity. J Ethnopharmacol 1989; 26: 1-55.

10. Luc G, Fruchart JC. Oxidation of lipoproteins and atherosclerosis. American J Clin Nutr 1991; 53: 2065–95.

Table 1. Effect of *Cassia auriculata* on plasma blood glucose level on different days (0day, 1st day, 3rd day, 5th day and 7th day) in normal and experimental animals:

Sr. No.	Group	Blood Glucose Level (mg/dl) C.A.				
		O day	1st day	3rd day	5th day	7th day
1	Normal	79.34± 3.705	79.80 ± 3.596	78.80 ± 3.42	80.34 ± 3.923	79.73 ± 3.473
2	STZ	248.5 ± 10.20***	224.7 ± 17.43***	232.4 ± 16.32***	229.5 ± 9.83***	204.2 ± 17.68***
3	Metformin	247.2 ± 12.93	171.0 ± 18.0	126.4 ± 1.32***	101.6 ± 6.69***	86.01 ± 7.14***
4	CA. Aq.	225.4 ± 19.98	190.1 ± 26.14	143.6 ± 7.862***	114.0 ± 9.174***	99.54 ± 6.23***
5	CA. Alc.	228.0 ± 23.23	232.0 ± 2.367	156.4 ± 14.76**	131.2 ± 8.956***	93.19 ± 14.81***
6	CA. Pet Ether	195.0 ± 20.06	196.8 ± 23.13	141.8 ± 7.71***	110.8 ± 8.088***	89.57 ± 14.60***
7	CA. CHCL3	217.8 ± 12.38	218.1 ± 18.96	188.5 ± 18.98	234.4 ± 16.177	199.1 ± 6.278

Values are expressed as Means ± SEM for six rats in each group

One way ANOVA followed by Dunnett's test.

STZ is compared with normal by using unpaired t-test

*P<0.05, **P<0.01, ***P<0.001 compared with STZ

Table 2. Effect of *Cassia auriculata* on plasma glucose concentration at different intervals (0hr, 1hr, 3hr, 5hr, and 7hr) during an OGTT in normal experimental animals:

Sr. No.	Group	Blood Glucose Level (mg/dl) C.A.				
		O hr.	1st hr.	3rd hr.	5th hr.	7th hr.
1	Normal	79.73 ± 3.473	94.07 ± 2.787	87.86 ± 2.965	81.31 ± 2.785	79.69 ± 2.202
2	STZ	204.2 ± 17.68***	250.9 ± 10.23***	241.3 ± 8.399***	236.2 ± 6.73***	233.9 ± 6.860***
3	Metformin	86.01 ± 7.14***	135.5 ± 17.68***	98.29 ± 8.094***	89.88 ± 4.808***	80.39 ± 3.88***
4	CA. Aq.	99.54 ± 6.23***	141.1 ± 8.34***	118.1 ± 4.358***	119.3 ± 4.567***	112.4 ± 5.654***
5	CA. Alc.	93.19 ± 14.81***	150.7 ± 8.879***	163.7 ± 30.15**	119.7 ± 10.88***	113.9 ± 13.91***
6	CA. Pet Ether	89.57 ± 14.60***	165.6 ± 20.80***	139.8 ± 8.039***	108.9 ± 9.22***	93.32 ± 6.669***
7	CA. CHCL3	199.1 ± 6.278	178.7 ± 11.76**	159.7 ± 16.11**	180.4 ±23.39**	193.7 ± 12.92*

Values are Means ± SEM for six rats in each group

One way ANOVA followed by Dunnett's test.

STZ is compared with normal by using unpaired t-test

*P<0.05, **P<0.01, ***P<0.001 compared with STZ

Table No. 3 Effect of *Cassia auriculata* and changes in plasma levels of cholesterol, triglyseride and HDL-cholesterol in normal and experimental animal:

Sr. No.	Treatment	Cholesterol	Triglyceride	HDL-Cholesterol
1	Normal	19.52 ± 0.6791	76.04 ± 1.473	26.60 ±2.542
2	STZ	65.08 ± 5.489***	123.0 ± 3.027***	19.13 ± 0.6223*
3	Metformin	28.34 ± 2.616**	79.75 ± 4.386***	39.39 ± 2.74*
4	Aq. CA	38.97 ± 1.942*	71.46 ± 3.715***	32.41 ± 2.64***
5	Alc. CA	33.29 ± 5.075*	69.35 ± 6.51***	30.27 ± 3.839**
6	CHCL3 CA	72.76 ± 6.195	105.6 ± 3.014*	21.33 ± 0.9921
7	Pet. Ether CA	67.34 ± 13.52	107.5 ± 4.835	22.29 ±1.402

Values are Means ± SEM for six rats in each group

One way ANOVA followed by Dunnett's test.

STZ is compared with normal by using unpaired t-test

*P<0.05, **P<0.01, ***P<0.001 compared with STZ

Figure No.1: Effect of *C. auriculata* on blood glucose level:

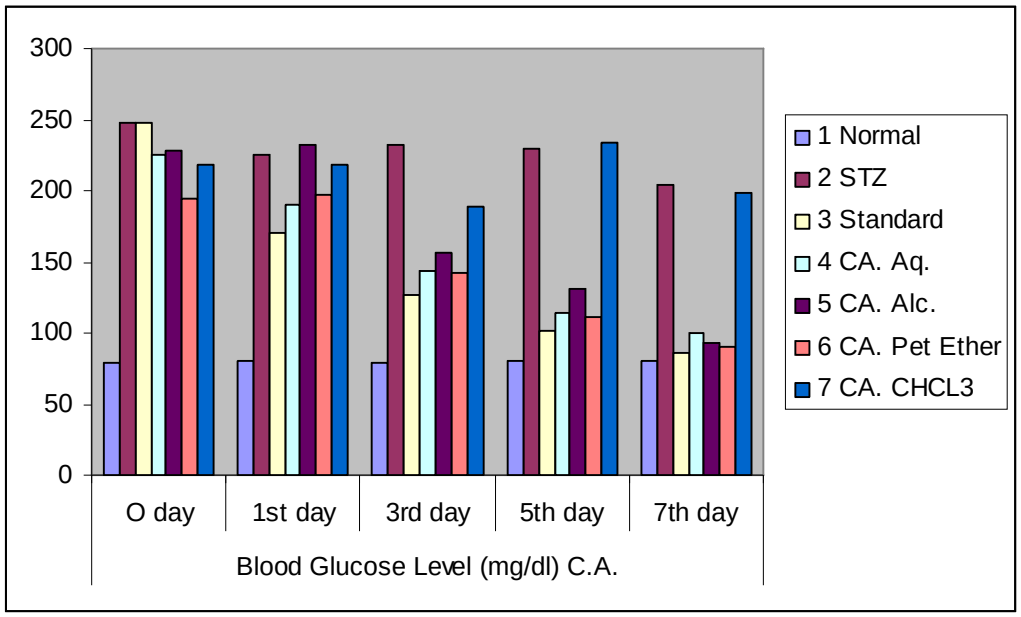


Figure No. 2: Effect of *C. auriculata* on OGTT:

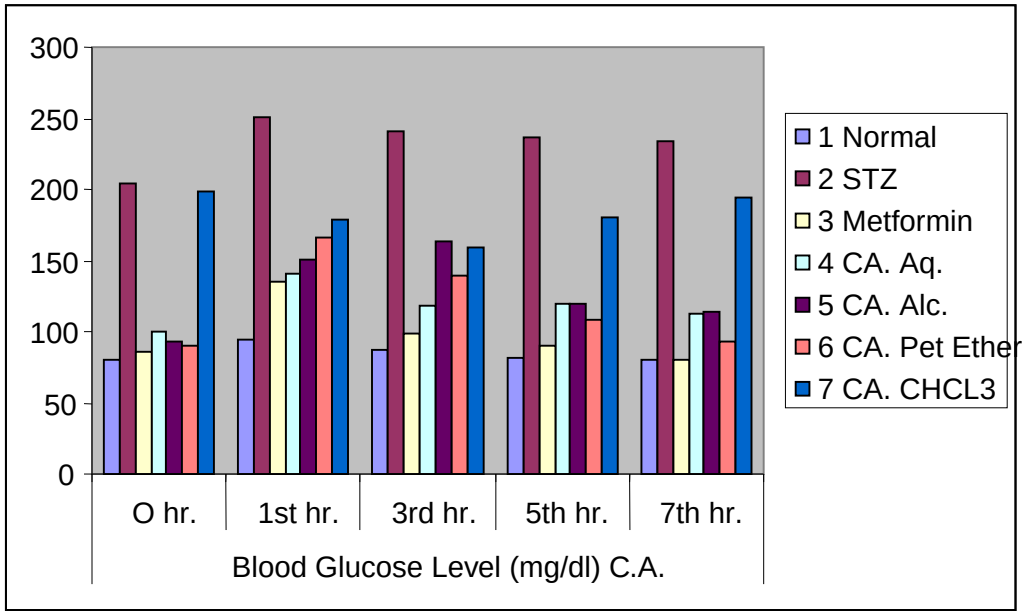
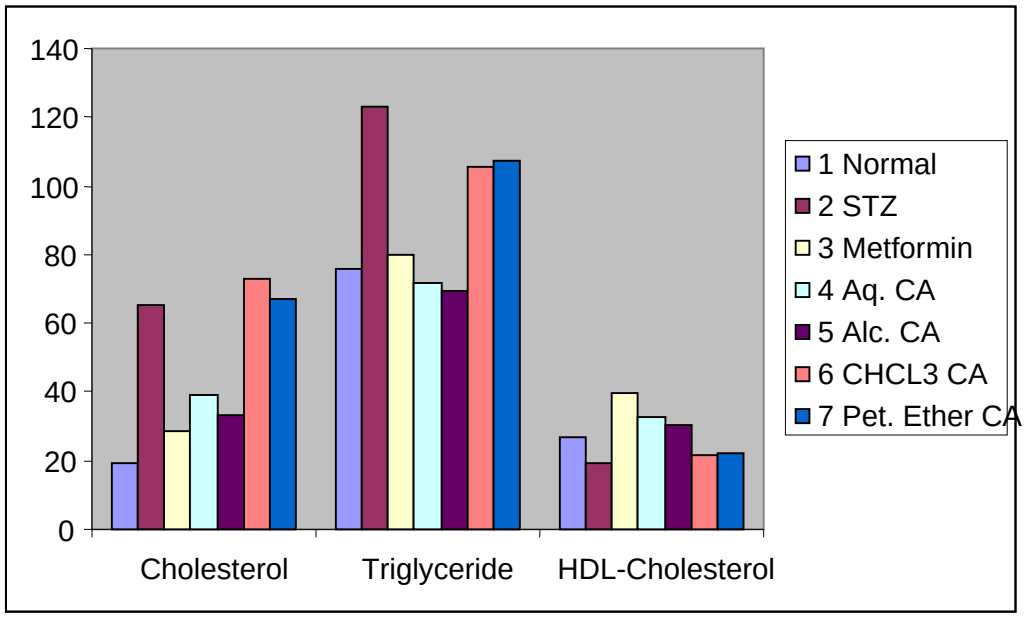


Figure No.3: Effect of *Cassia auriculata* on fasting lipid profile:



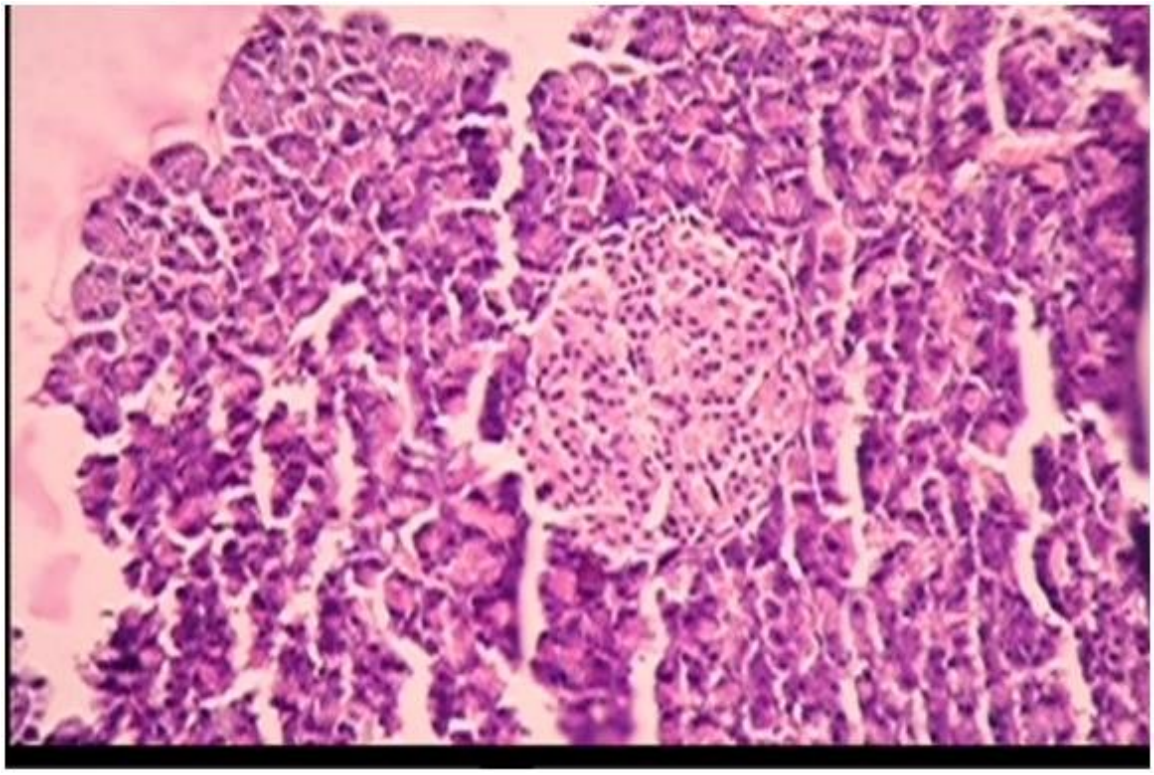


Figure A: Normal

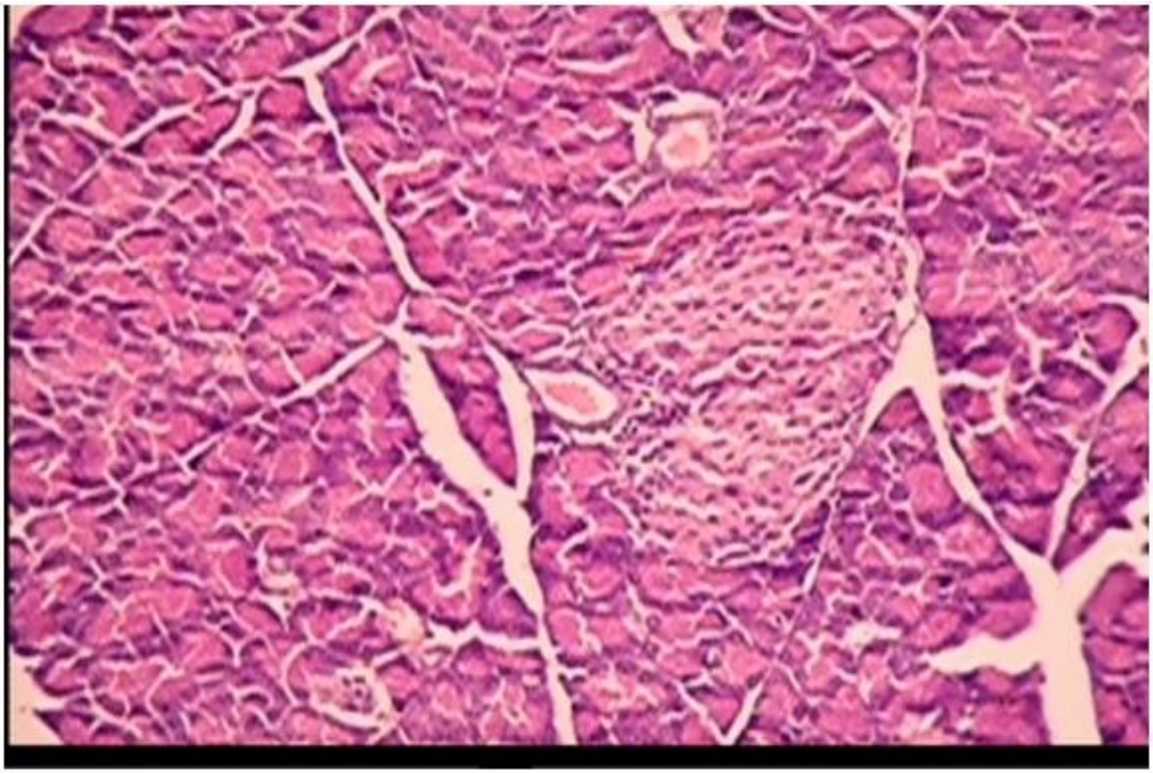


Figure B: STZ induced diabetic

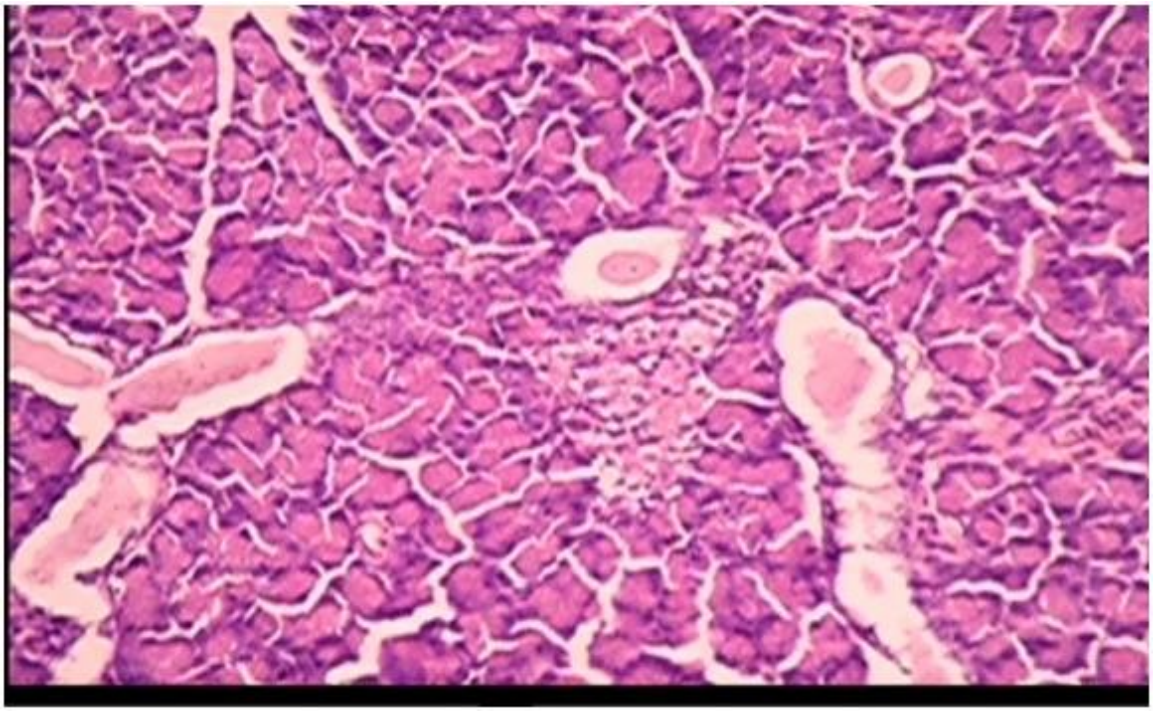


Figure C: Diabetic treated with 300 mg/kg bw aqueous extract

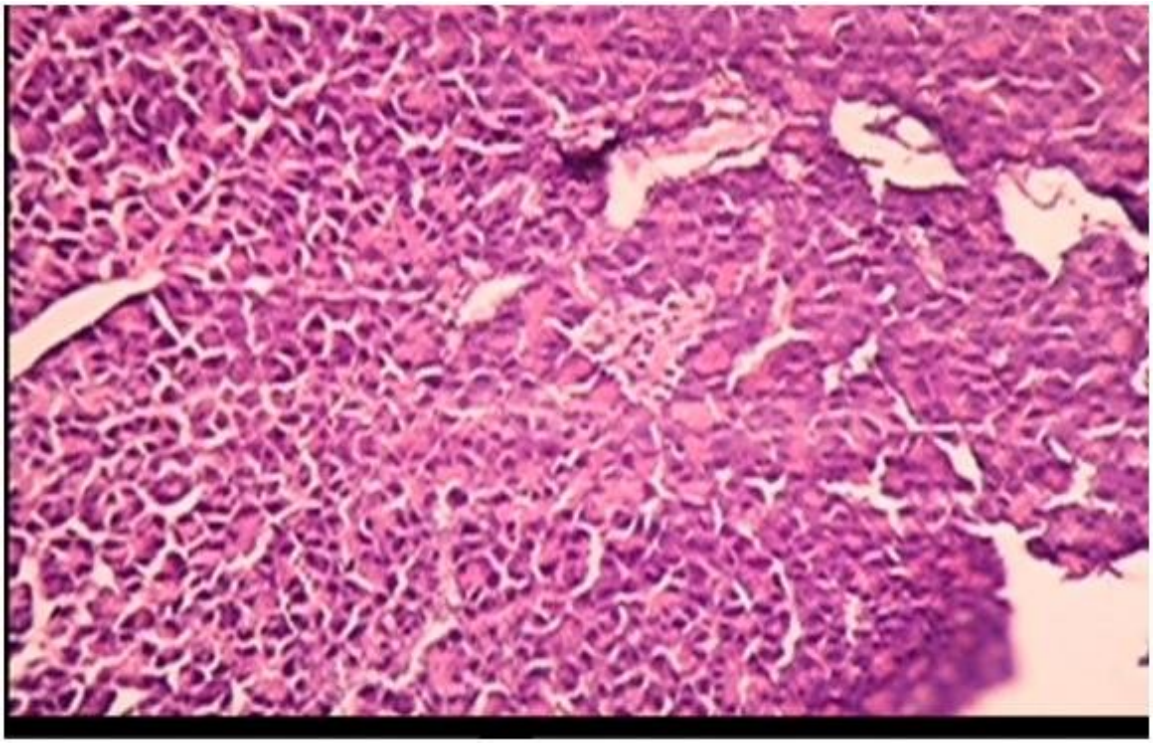


Figure D: Diabetic treated with 300 mg/kg bw alcoholic extract

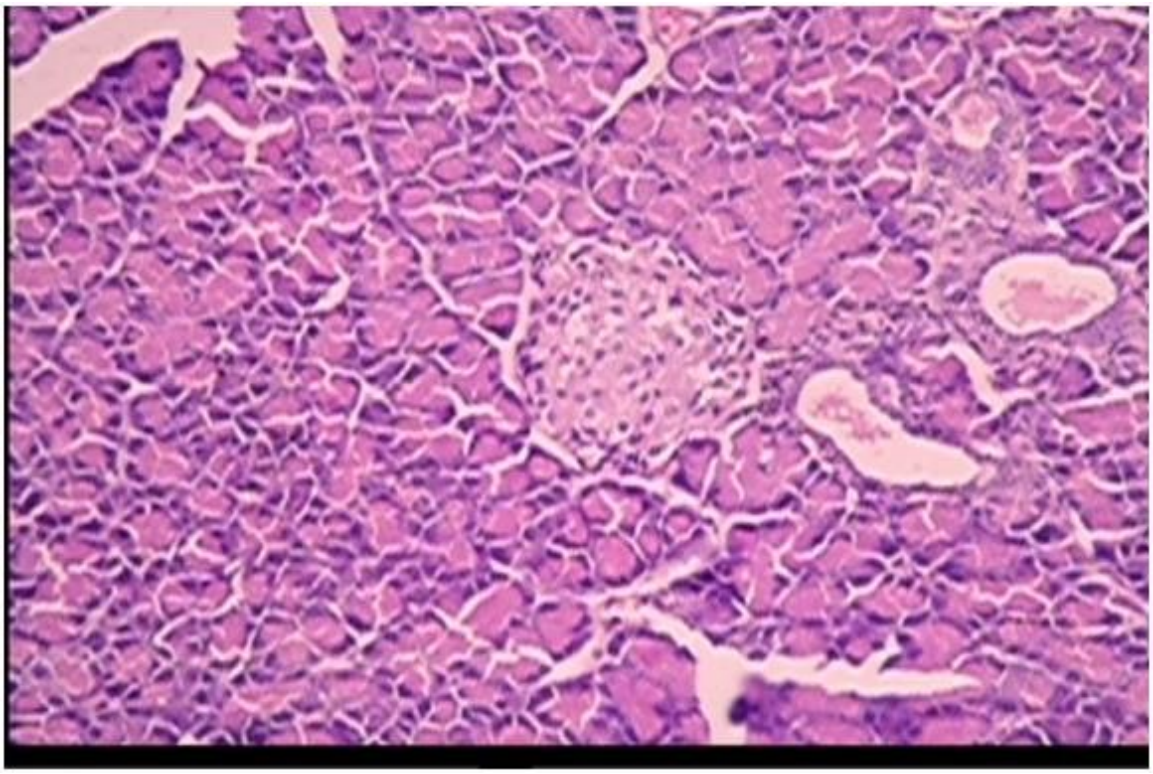


Figure E: Diabetic treated with 300 mg/kg bw pet.ether fraction

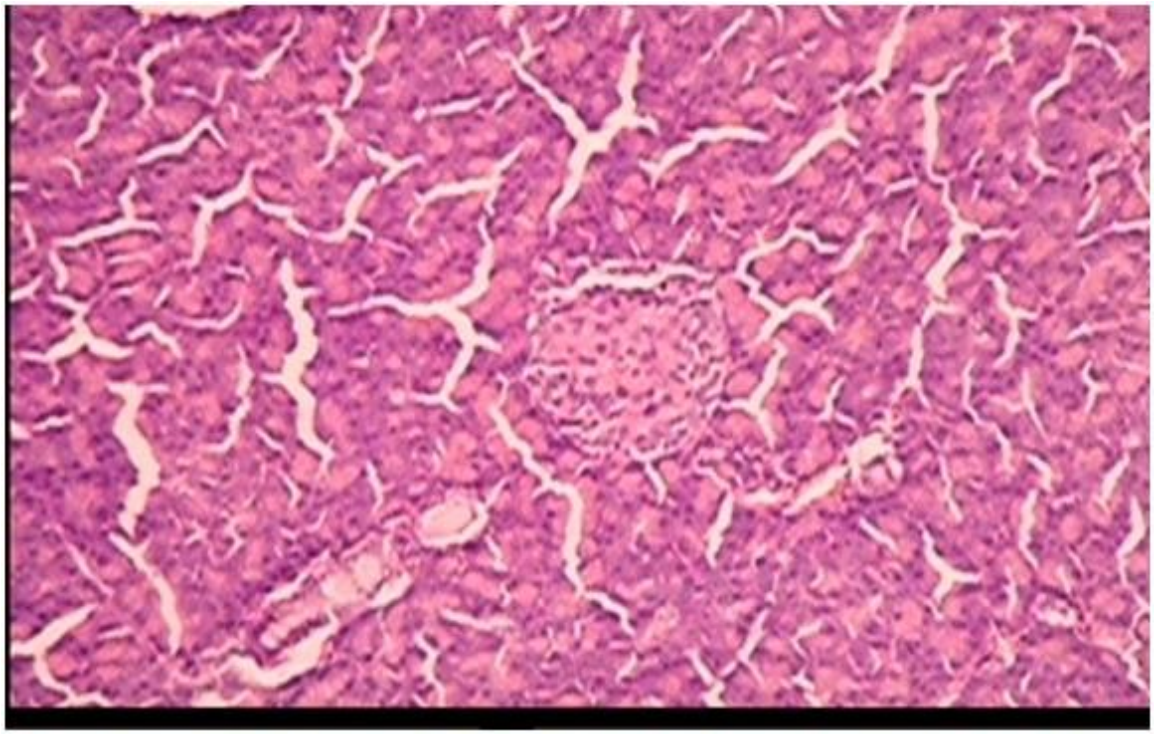


Figure F: Diabetic treated with 250 mg/kg b.w metformin